UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

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MEMORANDUM

SUBJECT: Human Health Assessment and Review of the Alternative Manufacturing Processes submitted by Verily Life Sciences, LLC and MosquitoMate Inc., for *Wolbachia pipientis* wAlbB *Ae. aegypti* EUP 89668-EUP-3. Decision #525795, DP# (438208).

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ACTION REQUESTED: Review the Alternative Manufacturing Process submitted by Verily Life Sciences, LLC and MosquitoMate Inc., for *Wolbachia pipientis* wAlbB, WB1 strain *Ae.aegypti* EUP 89668-EUP-3 and provide a new human health risk assessment.

1.0 BACKGROUND:

BPPD is conducting a review of an amendment to extend the experimental use permit (EUP), 89668-EUP-3. Given the extension expansion of releases of wAlbB *Ae. aegypti* expected with this amendment coupled with the significant public health importance of controlling *Ae. aegypti* which vector Zika and other arboviruses, the agency is conducting a new, updated human health effects risk assessment that incorporates new information from the manufacturing processes including gender separation methods.

2.0 EXPANDED RELEASES OF wAlbB *Ae. aegypti*:

Wolbachia pipientis belongs to the alpha subgroup of Proteobacteria, order Rickettsiales. It was first discovered in the mosquito *Culex pipiens* (Hertig & Wolbach, 1924). *W. pipientis* is an obligate intracellular bacterium that is commonly found in 65% of insects (Hilgenboecker et al. 2008), including mosquitoes in some geographic regions of the world, and does not survive and cannot be cultured outside of hosts. *Wolbachia* have been divided into eight different clades and *W. pipientis* wAlbB strain is categorized in the B clade (Dobson et al., 2004). Clades C and D occur in filarial nematodes and the B clade in mosquitoes are genetically different and incompatible (Baldo & Werren 2007, Hotopp et al. 2006, Lo et al. 2007, Werren et al. 2008).

W. pipientis has been introduced in *Ae. aegypti* through microinjection and interspecific breeding and is considered a microbial pest control agent (Xi et al. 2005a, Xi et al. 2005b). The presence of different *Wolbachia* strains in a mating can cause cytoplasmic incompatibility and karyogamy failure in the zygote. No offspring are produced when males carry *W. pipientis* are introduced into a population of mosquitoes that do not have *Wolbachia* present or carry different strains of *Wolbachia* resulting in greatly reduced reproduction. Cytoplasmic incompatibility arises because of asynchrony between the maternal and paternal pronucleus during mitosis. Due to this effect on egg hatch, male mosquitoes carrying the wAlbB strain of *Ae.aegypti* are released to mate with wild females already present in release areas to reduce *Ae.aegypti* mosquito population numbers.

The amendment extension includes the following:

- continue to release males in Fresno County, CA across 4 sites with an increase from a total of 4,800,000 (combined 2016 and 2017) to 192,000,000 (combined 2017 and 2018) male wAlbB *Ae. aegypti* released with a total of 2,664 acres (combined 2017 and 2018) used for release and monitoring.

- new releases of a total of 96,000,000 (combined 2017 and 2018) male wAlbB *Ae. aegypti* at 2 main locations with a total of 2,560 acres (combined 2017 and 2018) used for release and monitoring in Lee County, FL.

- new releases of a total of 192,000,000 (combined 2017 and 2018) male wAlbB *Ae. aegypti*

at 17 sites with a total of 6,400 acres (combined 2017 and 2018) used for release and monitoring in Harris County, TX.

- new releases of a total of 192,000,000 (combined 2017 and 2018) male wAlbB *Ae. aegypti* at 4 sites with a total of 3,200 acres (combined 2017 and 2018) used for release and monitoring in Miami-Dade County, FL.

3.0 EXPOSURE/RISK CHARACTERIZATION

Under the guidance of the National Academies of Sciences (NAS), EPA's Office of Pesticide Programs uses risk assessment to evaluate pesticides. Risk assessment most often includes evaluation of hazard and exposure using the basic equation: risk = hazard x exposure. In some cases, a pesticide may have no human health hazard or have no expected human exposure. In these cases, the pesticide does not exhibit human health risk.

The application of wAlbB *Ae. aegypti* as a pesticide under EUP 89668-EUP-3 is intended for males. Male mosquitoes do not bite and therefore do not expose humans to *W. pipientis*. Female mosquitoes take blood meals (i.e., bite) in order to have the nutrients needed to produce mosquito eggs. Therefore, the evaluation of the unintended release of females is the critical factor for the purposes of exposure assessment.

3.1 SUMMARY OF MOSQUITO GENDER SEPARATION PROCESSES

Both MosquitoMate and Verily submitted Alternative Manufacturing Processes as part of the amendment extension, including the rearing of the mosquitoes, details of the mosquito gender separation process, and quality control procedures to ensure the absence of medically important arboviruses. EPA's review of these can be found in Appendix 1 and the Data Evaluation Records (U.S. EPA 2017a, U.S. EPA 2017b).

3.1.1 MosquitoMate, Inc.

Larval mosquitoes are reared using standard techniques. In order to separate out female mosquitoes, pupae are sieved by a mechanical sorting device based on size because females in the pupal stage are larger than males. Two visual checks are performed by trained personnel after sieving to remove any residual females prior to shipment. The estimated female contamination rate is considered to be 1 female per 250,000 males released based on data provided.

3.1.2 Verily

Larval mosquitoes are reared using standard techniques. Once they pupate, they are mechanically sieved to remove females. Each newly emerged adult mosquito is isolated and individually photographed within 40 hours of emergence. The digital images are examined using a custom computer vision system and classification algorithm to confirm the mosquitoes are male and to identify any residual adult females or mosquitoes whose sex is ambiguous.

The overall female contamination rate was calculated by Verily to be equal to 0.00039% or 1 female per 258,014 males. This estimate of the female contamination rate was produced by actually adding females to the visual algorithm sorting system to detect how often a female will be missed by the visual algorithm. This approach was necessary because during the routine batch runs which includes mechanical sieving and the visual algorithm sorting system, no female has ever been detected.

3.1 CONCLUSION ON EXPOSURE/RISK CHARACTERIZATION

The mosquito gender separation processes used to produce male mosquitoes for release under this experimental use permit amendment/extension are highly efficient. Given the potential release rates from the MosquitoMate and Verily manufacturing methods of 1 female released per 250,000 males, there is negligible exposure to female wAlbB *Ae. aegypti* mosquitoes, and therefore the human health risk is negligible.

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U.S. EPA 2017b: Memo from Milutin S. Djurickovic to John L. Kough Verily Life Sciences, LLC, Manufacturing Process Data Evaluation Record MRID 50269702 for *Wolbachia pipientis* wAlbB *Ae. aegypti* EUP 89668-EUP-3.

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PAGES 6-8 (b)(4) Manufacturing Information Claimed confidential by submitter*